Claims

1. A nucleic acid molecule that comprises or that encodes, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, and a second base-paired region, wherein said first and second base-paired regions are base-paired to each other.

2. The nucleic acid molecule of claim 1, further comprising a second region of interest downstream of said second base-paired region, wherein said first and second regions of interest are based-paired to each other.

3. The nucleic acid molecule of claim 2, wherein said first and second regions of interest differ in length.

4. The nucleic acid molecule of claim 2, wherein said first region of interest has substantial identity to a region of a target gene and said second region of interest has substantial complementarity to said target gene, and wherein said nucleic acid molecule inhibits expression of said target gene in a cell.

5. The nucleic acid molecule of claim 4, wherein said first region of interest has substantial identity to a region of two or more target genes, and said second region of interest has substantial complementarity to said region of said two or more target genes, and wherein said nucleic acid molecule inhibits expression of said two or more target genes in a cell.

6. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises deoxyribonucleotides, ribonucleotides, or a mixture thereof.

7. The nucleic acid molecule of claim 4, wherein said target gene is a nucleic acid molecule associated with a disease or disorder, a bacterial infection, a viral infection, a yeast infection, or double-stranded ribonucleic acid (dsRNA)-mediated toxicity, or encodes a bacterial polypeptide, a viral polypeptide, a yeast polypeptide, a polypeptide associated with a disease or disorder, or a polypeptide associated with double-stranded ribonucleic acid (dsRNA)-mediated toxicity.

8. The nucleic acid molecule of claim 7, wherein said polypeptide associated with a disease or a disorder is a cancer-causing polypeptide.

9. The nucleic acid molecule of claim 7, wherein said polypeptide associated with dsRNA-mediated toxicity is a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide.

10. The nucleic acid molecule of any of preceeding claims 1-9, wherein said first region of interest is at least 1 to 1000 nucleotides.

11. The nucleic acid molecule of any of preceeding claims 1-10, wherein said first region of interest is at least 1 to 400 nucleotides.

12. The nucleic acid molecule of any of preceeding claims 1-11, wherein said first region of interest is at least 1 to 300 nucleotides.

13. The nucleic acid molecule of any of preceeding claims 1-12, wherein said first region of interest is at least 1 to 200 nucleotides.

14. The nucleic acid molecule of any of preceeding claims 1-13, wherein said first region of interest is at least 1 to 100 nucleotides.

15. The nucleic acid molecule of any of preceeding claims 1-14, wherein said first region of interest is at least 1 to 50 nucleotides.

16. The nucleic acid molecule of any of preceeding claims 1-15, wherein said first region of interest is at least 19 to 26 nucleotides.

17. The nucleic acid molecule of any of preceeding claims 1-15, wherein said first region of interest is at least 15 to 25 nucleotides.

18. The nucleic acid molecule of any of preceeding claims 1-15, wherein said first region of interest is at least 5 to 15 nucleotides.

19. The nucleic acid molecule of any of preceeding claims 2-18, wherein said second region of interest is at least 1 to 1000 nucleotides.

20. The nucleic acid molecule of any of preceeding claims 2-19, wherein said second region of interest is at least 1 to 400 nucleotides.

21. The nucleic acid molecule of any of preceeding claims 2-20, wherein said second region of interest is at least 1 to 300 nucleotides.

22. The nucleic acid molecule of any of preceeding claims 2-21, wherein said second region of interest is at least 1 to 200 nucleotides.

23. The nucleic acid molecule of any of preceeding claims 2-22, wherein said second region of interest is at least 1 to 100 nucleotides.

24. The nucleic acid molecule of any of preceeding claims 2-23, wherein said second region of interest is at least 1 to 50 nucleotides.

25. The nucleic acid molecule of any of preceeding claims 2-24, wherein said second region of interest is at least 19 to 26 nucleotides.

26. The nucleic acid molecule of any of preceeding claims 2-24, wherein said second region of interest is at least 15 to 25 nucleotides.

27. The nucleic acid molecule of any of preceeding claims 2-24, wherein said second region of interest is at least 5 to 15 nucleotides.

28. The nucleic acid molecule of any of preceeding claims 1-27, wherein said first base-paired region is at least 1 to 1000 nucleotides

29. The nucleic acid molecule of any of preceeding claims 1-28, wherein said first base-paired region is at least 1 to 400 nucleotides.

30. The nucleic acid molecule of any of preceeding claims 1-29, wherein said first base-paired region is at least 1 to 300 nucleotides.

31. The nucleic acid molecule of any of preceeding claims 1-30, wherein said first base-paired region is at least 1 to 200 nucleotides.

32. The nucleic acid molecule of any of preceeding claims 1-31, wherein said first base-paired region is at least 1 to 100 nucleotides.

33. The nucleic acid molecule of any of preceeding claims 1-32, wherein said first base-paired region is at least 1 to 50 nucleotides.

34. The nucleic acid molecule of any of preceeding claims 1-33, wherein said first base-paired region is at least 19 to 26 nucleotides.

35. The nucleic acid molecule of any of preceeding claims 1-33, wherein said first base-paired region is at least 15 to 25 nucleotides.

36. The nucleic acid molecule of any of preceeding claims 1-33, wherein said first base-paired region is at least 5 to 15 nucleotides.

37. The nucleic acid molecule of any of preceeding claims 1-36, wherein said second base-paired region is at least 1 to 1000 nucleotides

38. The nucleic acid molecule of any of preceeding claims 1-37, wherein said second base-paired region is at least 1 to 400 nucleotides.

39. The nucleic acid molecule of any of preceeding claims 1-38, wherein said second base-paired region is at least 1 to 300 nucleotides.

40. The nucleic acid molecule of any of preceeding claims 1-39, wherein said second base-paired region is at least 1 to 200 nucleotides.

41. The nucleic acid molecule of any of preceeding claims 1-40, wherein said second base-paired region is at least 1 to 100 nucleotides.

42. The nucleic acid molecule of any of preceeding claims 1-41, wherein said second base-paired region is at least 1 to 50 nucleotides.

43. The nucleic acid molecule of any of preceeding claims 1-42, wherein said second base-paired region is at least 19 to 26 nucleotides.

44. The nucleic acid molecule of any of preceeding claims 1-42, wherein said second base-paired region is at least 15 to 25 nucleotides.

45. The nucleic acid molecule of any of preceeding claims 1-42, wherein said second base-paired region is at least 5 to 15 nucleotides.

46. The nucleic acid molecule of any of preceeding claims 1-45, wherein said loop region is at least 1 to 1000 nucleotides

47. The nucleic acid molecule of any of preceeding claims 1-46, wherein said loop region is at least 1 to 400 nucleotides.

48. The nucleic acid molecule of any of preceeding claims 1-47, wherein said loop region is at least 1 to 300 nucleotides.

49. The nucleic acid molecule of any of preceeding claims 1-48, wherein said loop region is at least 1 to 200 nucleotides.

50. The nucleic acid molecule of any of preceeding claims 1-49, wherein said loop region is at least 1 to 100 nucleotides.

51. The nucleic acid molecule of any of preceeding claims 1-50, wherein said loop region is at least 1 to 50 nucleotides.

52. The nucleic acid molecule of any of preceeding claims 1-51, wherein said loop region is at least 19 to 26 nucleotides.

53. The nucleic acid molecule of any of preceeding claims 1-51, wherein said loop region is at least 15 to 25 nucleotides.

54. The nucleic acid molecule of any of preceeding claims 1-51, wherein said loop region is at least 5 to 15 nucleotides.

55. A pharmaceutical composition comprising the nucleic acid molecule of claim 1 and a pharmaceutically acceptable carrier or diluent.

56. A pharmaceutical composition comprising a DNA plasmid construct that encodes the nucleic acid molecule of any of preceeding claims 1-54, wherein said nucleic acid molecule comprises, at the 5' end, a promoter that is operably linked to said nucleic acid molecule and which enables transcription of said nucleic acid molecule, and wherein said nucleic acid molecule encodes, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, and a second base-paired region, wherein said first and second base-paired regions are base-paired to each other, and wherein transcription of said nucleic acid molecule produces a RNA hairpin.

57. A method for generating an RNA hairpin comprising transcribing the nucleic acid molecule of any of preceeding claims 1-54 in a host cell that has been transformed with said nucleic acid molecule, wherein said nucleic acid molecule comprises, at the 5' end, a promoter that is operably linked to said nucleic acid molecule and which enables transcription of said nucleic acid molecule, and wherein said nucleic acid molecule encodes, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, and a second base-paired region, wherein said first and second base-paired regions are base-paired to each other, wherein transcription of said nucleic acid molecule produces a RNA hairpin.

58. The method of claim 57, wherein said nucleic acid molecule further encodes a second region of interest downstream of said second base-paired region, wherein said first and second regions of interest are base-paired to each other.

59. The method of claim 58, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 5' end of said first region of interest extends beyond the 3' end of said second region of interest.

60. The method of claim 58, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 3' end of said second region of interest extends beyond the 5' end of said first region of interest.

61. The method of claim 59, wherein said non-overlapping region of said partial RNA hairpin is extended *in vivo* by an RNA-dependent RNA polymerase.

62. The method of claim 61, wherein said RNA-dependent RNA polymerase is endogenous to said host cell.

63. The method of claim 61, wherein said RNA-dependent RNA polymerase is exogenous to said host cell and is provided to said host cell.

64. A method for inhibiting the expression of a target gene in a cell, said method comprising administering to a subject in need thereof, the nucleic acid molecule of any of preceeding claims 1-54 that comprises or that encodes an RNA hairpin, wherein said RNA hairpin comprises, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, a second base-paired region, and a second region of interest, wherein said first and second base-paired regions are base-paired to each other, and wherein said administering inhibits or reduces expression of a target gene, relative to expression of said target gene in a subject not administered said nucleic acid molecule.

65. The method of claim 64, wherein said first and second regions of interest are the same or different lengths.

66. The method of claim 65, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 5' end of said first region of interest extends beyond the 3' end of said second region of interest.

67. The method of claim 65, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 3' end of said second region of interest extends beyond the 5' end of said first region of interest.

68. The method of claim 66, wherein said non-overlapping region of said partial RNA hairpin is extended *in vivo* by an RNA-dependent RNA polymerase.

69. The method of claim 68, wherein said RNA-dependent RNA polymerase is endogenous to said host cell.

70. The method of claim 68, wherein said RNA-dependent RNA polymerase is exogenous to said host cell and is provided to said host cell.

71. The method of claim 64, wherein said first region of interest has substantial identity to a region of said target gene and said second region of interest has substantial complementarity to said target gene, and wherein said nucleic acid molecule inhibits expression of said target gene in a cell of said subject.

72. The method of claim 71, wherein said first region of interest has substantial identify to a region of two or more target genes, and said second region of interest has substantial complementarity to said region of said two or more target genes, and wherein said nucleic acid molecule inhibits expression of said two or more target genes in a cell of said subject.

73. The method of claim 71, wherein said target gene is a nucleic acid molecule associated with a disease or disorder, a bacterial infection, a viral infection, a yeast infection, or double-stranded ribonucleic acid (dsRNA)-mediated toxicity, or encodes a bacterial polypeptide, a viral polypeptide, a yeast polypeptide, a polypeptide associated with a disease or disorder, or a polypeptide associated with double-stranded ribonucleic acid (dsRNA)-mediated toxicity.

74. The method of claim 73, wherein said polypeptide associated with a disease or a disorder is a cancer-causing polypeptide.

75. The method of claim 73, wherein said polypeptide associated with dsRNA-mediated toxicity is a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide.

76. A method for treating or preventing infection, said method comprising administering to a subject in need thereof, the nucleic acid molecule of any of preceeding claims 1-54 that comprises or that encodes an RNA hairpin, wherein said RNA hairpin comprises, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, a second base-paired region, and a second region of interest, wherein said first and second base-paired regions are base-paired to each other, and wherein said administering inhibits or reduces expression of a target gene, relative to expression of said target gene in a subject not administered said nucleic acid molecule, wherein said target gene is a bacterial, viral, or yeast gene that encodes a polypeptide required for infection, replication, pathogenesis, or survival of said bacteria, virus, or yeast in said subject.

77. The method of claim 76, wherein said first and second regions of interest are the same or different lengths.

78. The method of claim 77, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 5' end of said first region of interest extends beyond the 3' end of said second region of interest.

79. The method of claim 77, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 3' end of said second region of interest extends beyond the 5' end of said first region of interest.

80. The method of claim 78, wherein said non-overlapping region of said partial RNA hairpin is extended *in vivo* by an RNA-dependent RNA polymerase.

81. The method of claim 80, wherein said RNA-dependent RNA polymerase is endogenous to said host cell.

82. The method of claim 80, wherein said RNA-dependent RNA polymerase is exogenous to said host cell and is provided to said host cell.

83. The method of claim 76, wherein said first region of interest has substantial identity to a region of said target gene and said second region of interest has substantial complementarity to said target gene, and wherein said nucleic acid molecule inhibits expression of said target gene in a cell of said subject.

84. The method of claim 83, wherein said first region of interest has substantial identify to a region of two or more target genes, and said second region of interest has substantial complementarity to said region of said two or more target genes, and wherein said nucleic acid molecule inhibits expression of said two or more target genes in a cell of said subject.

85. The method of claim 76 further comprising administering to a subject in need thereof, a second nucleic acid molecule that comprises or that encodes a second RNA hairpin, wherein said second RNA hairpin comprises, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, a second base-paired region, and a second region of interest, wherein said first and second base-paired regions are base-paired to each other, and wherein said administering inhibits or reduces expression of a second target gene, relative to expression of said second target gene in a subject not administered said second nucleic acid molecule, wherein said second target gene encodes a polypeptide associated with double-stranded ribonucleic acid (dsRNA)-mediated toxicity.

86. The method of claim 85, wherein said polypeptide associated with dsRNA-mediated toxicity is a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide.

87. The method of 86, wherein said first region of interest of said second nucleic acid molecule has substantial identity to a region of a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide and said second region of interest of said second nucleic acid molecule has substantial complementarity to said region of a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide.

88. A method for treating or preventing cancer, said method comprising administering to a subject in need thereof, an effective amount of a nucleic acid molecule that comprises or that encodes a RNA hairpin, wherein said RNA hairpin comprises, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, a second base-paired region, and a second region of interest, wherein said first and second base-paired regions are base-paired to each other, and wherein said administering inhibits or reduces expression of a target gene, relative to expression of said target gene in a subject not administered said nucleic acid molecule, and wherein said target gene encodes a polypeptide required for proliferation, maintenance, or survival of a cancer-causing cell.

89. The method of claim 88, wherein said first and second regions of interest are the same or different lengths.

90. The method of claim 89, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 5' end of said first region of interest extends beyond the 3' end of said second region of interest.

91. The method of claim 88, wherein the 5' end of said RNA hairpin comprising the first region of interest and the 3' end of said RNA hairpin comprising the second region of interest partially overlap to form a partial RNA hairpin having a non-overlapping region, wherein the 3' end of said second region of interest extends beyond the 5' end of said first region of interest.

92. The method of claim 90, wherein said non-overlapping region of said partial RNA hairpin is extended *in vivo* by an RNA-dependent RNA polymerase.

93. The method of claim 92, wherein said RNA-dependent RNA polymerase is endogenous to said host cell.

94. The method of claim 92, wherein said RNA-dependent RNA polymerase is exogenous to said host cell and is provided to said host cell.

95. The method of claim 88, wherein said first region of interest has substantial identity to a region of said target gene and said second region of interest has substantial complementarity to said target gene, and wherein said nucleic acid molecule inhibits expression of said target gene in a cell of said subject.

96. The method of claim 95, wherein said first region of interest has substantial identify to a region of two or more target genes, and said second region of interest has substantial complementarity to said region of said two or more target genes, and wherein said nucleic acid molecule inhibits expression of said two or more target genes in a cell of said subject.

97. The method of claim 88 further comprising administering to a subject in need thereof, a second nucleic acid molecule that comprises or that encodes a second RNA hairpin, wherein said second RNA hairpin comprises, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, a second base-paired region, and a second region of interest, wherein said first and second base-paired regions are base-paired to each other, and wherein said administering inhibits or reduces expression of a second target gene, relative to expression of said second target gene in a subject not administered said second nucleic acid molecule, wherein said second target gene encodes a polypeptide associated with double-stranded ribonucleic acid (dsRNA)-mediated toxicity.

98. The method of claim 97, wherein said polypeptide associated with dsRNA-mediated toxicity is a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide.

99. The method of 98, wherein said first region of interest of said second nucleic acid molecule has substantial identity to a region of a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide and said second region of interest of said second nucleic acid molecule has substantial complementarity to said region of a RNA-dependent protein kinase (PKR) polypeptide or an interferon-response associated polypeptide.

100. A method for treating, stabilizing, or preventing a disease, disorder, or infection in an animal, said method comprising introducing into said animal a first agent that provides to said animal the nucleic acid molecule of any of preceeding claims 1-54, wherein said first region of interest of said nucleic acid molecule has substantial sequence identity to a region of a target nucleic acid associated with said disease, disorder, or infection and specifically inhibits expression of said target nucleic acid molecule.

101. The method of claim 100, wherein said target nucleic acid molecule is associated with a pathogen.

102. The method of claim 101, wherein said pathogen is a virus, bacterium, yeast, or infectious agent.

103. A method for identifying a nucleic acid molecule that modulates a detectable phenotype in a cell, said method comprising the steps of:

(a) transforming a population of cells with a dsRNA expression library, wherein said library comprises a plurality of the nucleic acid molecule of any of preceeding claims 1-54, wherein said first region of interest of said plurality of nucleic acid molecule comprises a sequence having substantial identity to said nucleic acid molecule that modulates a detectable phenotype in a cell; and

wherein at least two cells of said population of cells are each transformed with a different nucleic acid molecule from said dsRNA expression library; and

(b) assaying for a modulation in said detectable phenotype, wherein said modulation identifies a nucleic acid molecule that is associated with said phenotype.

104. The method of claim 103, wherein said modulation in a detectable phenotype is a modulation in the function of a cell, a modulation in the biological activity of a polypeptide, or a modulation in the expression of a target nucleic acid molecule.

105. The method of claim 103, further comprising:

(c) identifying said nucleic acid molecule that modulates a detectable phenotype in a cell by amplifying said nucleic acid molecule and sequencing said amplified nucleic acid molecule.

106. The method of claim 103, wherein said dsRNA expression library comprises cDNAs derived from said cells.

107. A kit for the synthesis of an RNA hairpin, said kit comprising a dsRNA expression construct comprising a promoter that is operably linked to, and is effective for expression of, a first downstream coding sequence in a host cell, said first downstream coding sequence comprising, in 5' to 3' order, a first multiple cloning site (MCS) downstream of said promoter, a first base-paired region, a loop region, a second base-paired region, and a second MCS, wherein a first region of interest inserted into said first MCS and a second region of interest inserted into said second MCS is transcribed by said promoter, and, following transcription of said first downstream coding sequence, said first and second base-paired regions are base-paired to each other.

108. The kit of claim 107, wherein said kit further comprises a ribonucleic acid (RNA)-dependent RNA polymerase.

109. The kit of claim 107, wherein said kit further comprises a second dsRNA expression construct comprising a promoter that is operably linked to, and is effective for expression of, an RNA-dependent RNA polymerase in a host cell.

110. The kit of claim 107, wherein said dsRNA expression construct is bicistronic and further comprises a second promoter that is operably linked to, and is effective for expression of, a second downstream coding sequence, wherein said second downstream coding sequence comprises an RNA-dependent RNA polymerase that is co-expressed with said first downstream coding sequence.

111. The kit of claim 107, wherein said DNA plasmid construct further comprises a selectable drug resistance marker.

112. The kit of claim 111, wherein said selectable drug resistance marker is a zeomycin, kanamycin, an aminoglycoside, tetracycline, ampicillin, or hygromycin drug resistance gene.

113. The kit of any of preceeding claims 107, wherein said dsRNA expression construct comprises a DNA plasmid construct.

114. The kit of claim 109, wherein said second dsRNA expression construct comprises a DNA plasmid construct.

115. Use of a nucleic acid molecule that comprises or that encodes, in 5' to 3' order, a first region of interest, a first base-paired region, a loop region, and a second base-paired region, wherein said first and second base-paired regions are base-paired to each other, for the manufacture of a medicament for inhibiting expression of a target gene, for treating or preventing infection, or for treating or preventing cancer.

116. The use of claim 115, wherein said nucleic acid molecule further comprises a second region of interest, wherein said first region of interest has substantial identity to a region of a target gene and said second region of interest has substantial complementarity to said target gene, and wherein said nucleic acid molecule inhibits expression of said target gene in a cell.